specific lipoprotein.

46. A kit for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

monoclonal Apo-A-I antibody specifically immunoreactive with Apo A-I lipoproteins in human plasma; and

monoclonal Apo A-II antibody specifically immunoreactive with Apo A-II.

47. The kit of claim 46 wherein the monoclonal or recombinant antibody molecule specifically immunoreactive with a single specific lipoprotein or apolipoprotein is selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

Remarks

The Interview

Applicants and the undersigned greatly appreciated the examiner's cooperation and very helpful suggestions during the interview on August 19, 1999, at the Patent Office.

As indicated on the Interview Summary record, this application claims methods for determining the relative ratios of one apolipoprotein or lipopoprotein to another, which is not described in the prior art.

Amendments to the Claims

As discussed at the interview the claims in the related application, U.S.S.N. 08/765,324,

which were also drawn to a method for determining the relative ratio of apolipoproteins or lipoproteins to another apolipoprotein or lipoprotein, claims 15, 16, and 42, have been added to this application as new claims 39, 40 and 41 and cancelled from the '324. This should moot any double patenting issues. Claims to the reagents specific for use in these assays from the related '324 application, claims 28-30, have also been added to this application as new claims 42-47 (and the term "composition" replaced with the more accurate term "kit"). Claims to a method for making antibodies, claims 30-32, were cancelled. Where appropriate, the claims were amended to recite that the monoclonal antibodies were "specifically" immunoreactive.

U.S.S.N.: 08/970,045
Filed: November 13, 1997
Amendment

Allowance of claims 1-13, as amended, and new claims 39-47, is earnestly solicited. All claims as pending upon entry of this amendment are attached in an Appendix to facilitate review by the Examiner.

Respectfully submitted,

Patrea L. Pabst
Reg. No. 31,284

Date: August 26, 1999
ARNALL GOLDEN & GREGORY, LLP
2800 One Atlantic Place
1201 West Peachtree Street
Atlanta, GA 30309-3450
(404) 873-8794
(404) 873-8795 fax

CERTIFICATE OF FACSIMILE TRANSMISSION (37 CFR 1.8a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being facsimile transmitted to the Assistant Commissioner of Patents, Washington, D.C. 20231 on the date shown below.

Date: August 26, 1999

Jean Hicks

APPENDIX: Claims as Pending Upon Entry of the Amendment

1. (amended) The method for determining the relative ratio of LDL to HDL or at least two different apolipoproteins in a biological sample comprising:

immersing into the sample a solid phase material having separately immobilized thereon at least first and second monoclonal antibody molecules immunoreactive with a specific lipoprotein indicative of LDL or HDL or at least two different apolipoproteins;

allowing the monoclonal antibody molecules time to bind to the lipoprotein in the LDL and HDL or apolipoproteins in the sample;

removing the solid phase material containing the immobilized monoclonal antibody molecules;

determining the amount of LDL and HDL lipoprotein or at least two different apolipoproteins bound by the immobilized monoclonal antibody molecules,and

comparing the amount bound which is specific for LDL or HDL or each apolipoprotein in order to calculate the relative amounts of LDL and HDL or apolipoproteins.

2. The method of claim 1 wherein the antibody molecules immobilized on the solid phase material are immunoreactive with a lipoproteins selected from the group consisting of HDL, LDL, VLDL, and combinations thereof.

3. (amended) The method of claim 2 wherein the antibody is selected from the group consisting of recombinant antibodies and antibody fragments.

4. The method of claim 3, wherein the antibody is the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

5. The method of claim 3, wherein the antibody is a recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

6. (amended) The method of claim 1 wherein the amount of lipoprotein or lipid associating with apolipoprotein is determined by staining of the material bound to the immobilized antibody using a lipid stain.

7. The method of claim 6 wherein the lipid stain is selected from the group consisting of Sudan Red 7B, Oil Red O, and Sudan Black B.

8. The method of claim 6 wherein the lipoprotein lipid is stained prior to immersing the immobilized antibodies.

9. (amended) The method of claim 6, further comprising a third antibody immunoreactive with apolipoprotein wherein the third antibody is coupled to a protein stain and used to stain lipoprotein in the sample, prior to immersing into the sample the immobilized first antibodies which then bind to the stained second antibody-bound apolipoprotein.

10. The method of claim 1, wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

11. The method of claim 1, wherein the biological sample is selected from the group consisting of blood, plasma, and serum.

12. (amended) A method of determining the relative concentration of at least two different apolipoproteins in a biological sample comprising:

mixing a first and second monoclonal antibody molecules each immunoreactive with a specific apolipoprotein into the sample;

allowing the monoclonal antibody molecules to bind to the apolipoprotein in the sample,

immersing into the mixture third immobilized monoclonal antibody molecules immunoreactive with a second, distinct epitope of an apolipoprotein,

allowing the immobilized monoclonal antibody molecules to bind to the apolipoprotein,

detecting the presence of the apolipoprotein bound by both both monoclonal antibodies, and

determining the amount of each apolipoprotein bound by both monoclonal antibodies.

13. The method of claim 12 wherein the apolipoprotein is apolipoprotein Apo B-100.

Please cancel claims 30-32.

Please add new claims 39-47.

39. A method for determining the relative ratio of LDL to HDL in a biological sample comprising

determining the amount of LDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with low density lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein;

determining the amount of HDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with high density lipoprotein and not cross-reactive with low density lipoprotein and determining the amount of high density lipoprotein; and

determining the ratio of the amount of low density lipoprotein with the amount of high density lipoprotein.

40. A method for determining the relative ratio of VLDL to HDL in a biological sample comprising

determining the amount of VLDL in the sample by

determining the amount of Apo C-III present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the antibody reactive with Apo C-III with the biological sample to form complexes between the antibody and the Apo C-III containing lipoprotein particles,

contacting the Pan B antibody with the biological sample,

separating the complexed antibody-lipoprotein particles from the biological sample, and

determining the amount of Apo C-III associated with Apo B, which is the amount of Apo C-III present in VLDL in the sample; and

determining the amount of HDL in the sample by

determining the amount of Apo C-III present in the HDL in the sample by

providing Apo A-I monoclonal antibody specifically immunoreactive specifically with

Apo A-I,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the antibody reactive with Apo C-III with the biological sample to form complexes between the antibody and the Apo C-III containing lipoprotein particles,

contacting the anti-Apo A-I antibody with the biological sample,

separating the complexed antibody-lipoprotein particles from the biological sample,

determining the amount of Apo C-III associated with Apo A-I, which is the amount of Apo C-III present in HDL in the sample, and

determining the ratio of Apo C-III present in VLDL in the sample and Apo C-III present in HDL in the sample which is the ratio of VLDL to HDL,

wherein the VLDL and HDL are measured in the same sample using immobilized antibodies or measured by immunoprecipitation in separate samples.

41. A method for determining the relative ratio of VLDL to HDL comprising

determining the amount of VLDL in the sample by

determining the amount of Apo E present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody which specifically binds to Apo E associated predominantly with VLDL,

contacting the antibodies reactive with Apo E associated with VLDL with the biological sample to form complexes between the antibodies and Apo E containing particles,

contacting Pan B antibody with the biological sample, and

determining the amount of Apo E associated with Apo B which is the Apo E present predominantly in VLDL in the sample;

removing the complexed anti-Apo E:Pan B:Apo E containing particles by immobilization of the anti-Apo E antibodies or centrifugation of complexed particles;
and

determining the amount of HDL in the sample by

determining the amount of Apo E present in the HDL in the sample by

providing Apo A-I monoclonal antibody immunoreactive specifically with Apo A-I,

providing monoclonal antibody which binds to Apo E predominantly associated with HDL,

contacting the antibodies reactive with Apo E to the biological sample to form complexes between the antibodies and Apo E containing particles,

contacting Pan B antibody with the biological sample,

determining the amount of Apo E associated with Apo A-I, which is the amount of Apo E present in HDL in the sample, and

determining the ratio of Apo E present in VLDL in the sample and Apo E present in HDL in the sample which is the ratio of VLDL to HDL.

42. A kit for determining the relative ratio of VLDL to HDL comprising

Pan B antibody which is characterized by an equal binding and high affinity for all Apo

B-containing lipoproteins in human plasma,

monoclonal antibody specifically immunoreactive with Apo C-III, and

monoclonal Apo A-I antibody specifically immunoreactive with Apo A-I.

43. The kit of claim 42 wherein the monoclonal or recombinant antibody molecules specifically immunoreactive with a single specific lipoprotein or apolipoprotein are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

44. A kit for determining the relative ratio of VLDL to HDL comprising

Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

monoclonal antibody which predominantly binds to Apo E associated with VLDL ,

monoclonal Apo A-I antibody specifically immunoreactive with Apo A-I, and

monoclonal antibody which predominantly binds to Apo E in HDL.

45. The kit of claim 44 wherein the monoclonal or recombinant antibody molecules specifically immunoreactive with a single specific lipoprotein or apolipoprotein are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

46. A kit for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

monoclonal Apo-A-I antibody specifically immunoreactive with Apo A-I lipoproteins in human plasma; and

monoclonal Apo A-II antibody specifically immunoreactive with Apo A-II.

47. The kit of claim 46 wherein the monoclonal or recombinant antibody molecule specifically immunoreactive with a single specific lipoprotein or apolipoprotein is selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.